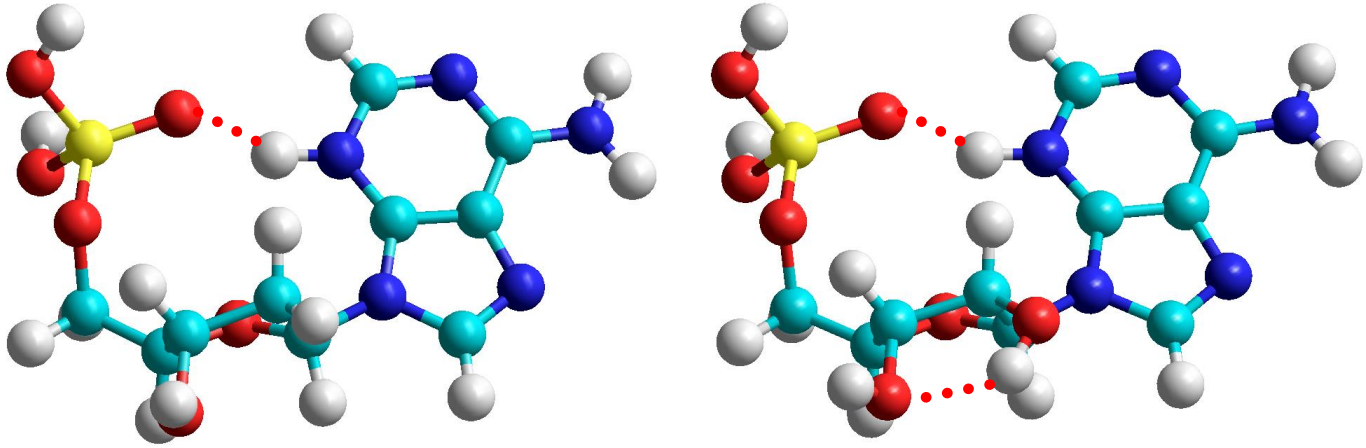
Gas-Phase Conformations and Energetics of Protonated 2'-Deoxyadenosine-5'-Monophosphate and Adenosine-5'-Monophosphate: IRMPD Action Spectroscopy and Theoretical Studies



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Giel Berden,[‡] Jos Oomens[‡§] and M. T. Rodgers[†]

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Introduction

- Importance of adenine nucleotides:
  - Adenine: high photostability, preserves genetic information
  - ADP, ATP: activate enzymatic transformations
  - cAMP: control the actions of peptide hormones
  - NAD, FAD: induce redox reactions

- Effects of protonation:
  - $A^+ \bullet C$ and $A^+ \bullet G$ base pairs
  - Isolated adenine: N1, most favorable protonation site
  - Adenine nucleosides: N3, allows syn orientation of adenine, and forms $N3H^+ \cdots O5'$ hydrogen-bonding interaction.
  - Protonation at N3: adenine nucleobase flipping.
  - Sugar moiety changes the protonation preferences.

Knowles, J. R. *Annu. Rev. Biochem.* **1989**, *58*, 195.
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Dodge-Kafka, K. L.; Kapiloff, M. S. *Eur. J. Cell Biol.* **2006**, *85*, 593.
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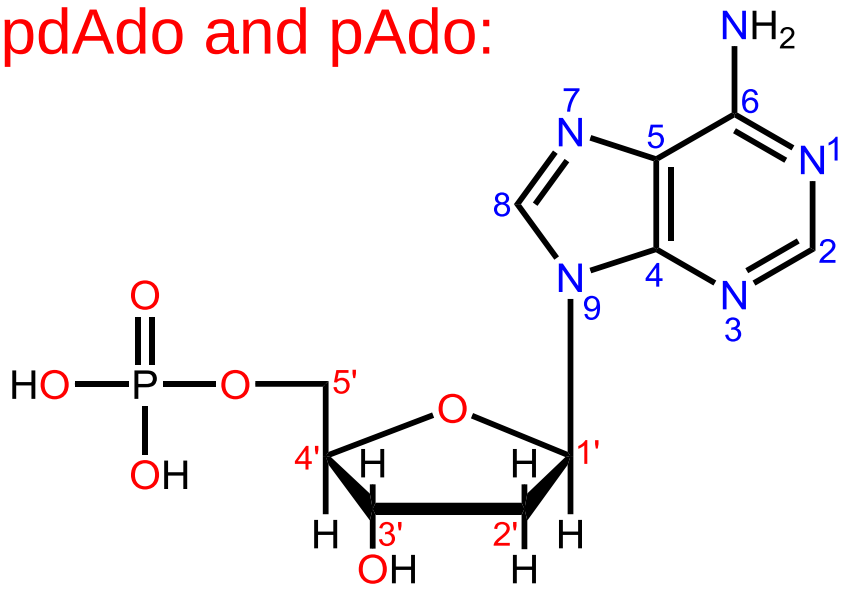
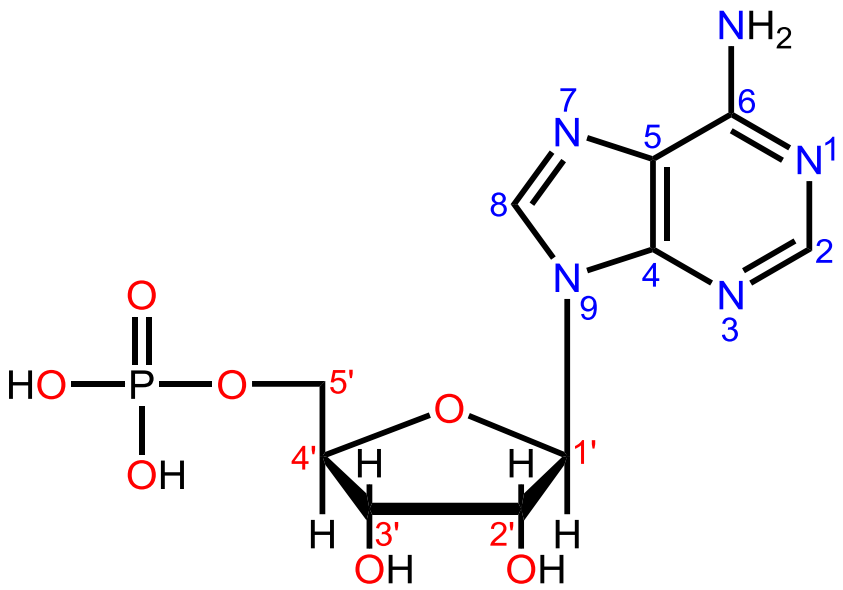
Russo, N.; Toscano, M.; Grand, A.; Jolibis, F. *J. Comput. Chem.* **1998**, *9*, 989.
Wu, R. R.; Yang, B.; Berden, G.; Oomens, J.; Rodgers, M. T. *J. Phys. Chem. B* **2015**, *119*, 2795.

Introduction

- pdAdo and pAdo:



2'-deoxyadenosine-5'-monophosphate



adenosine-5'-monophosphate

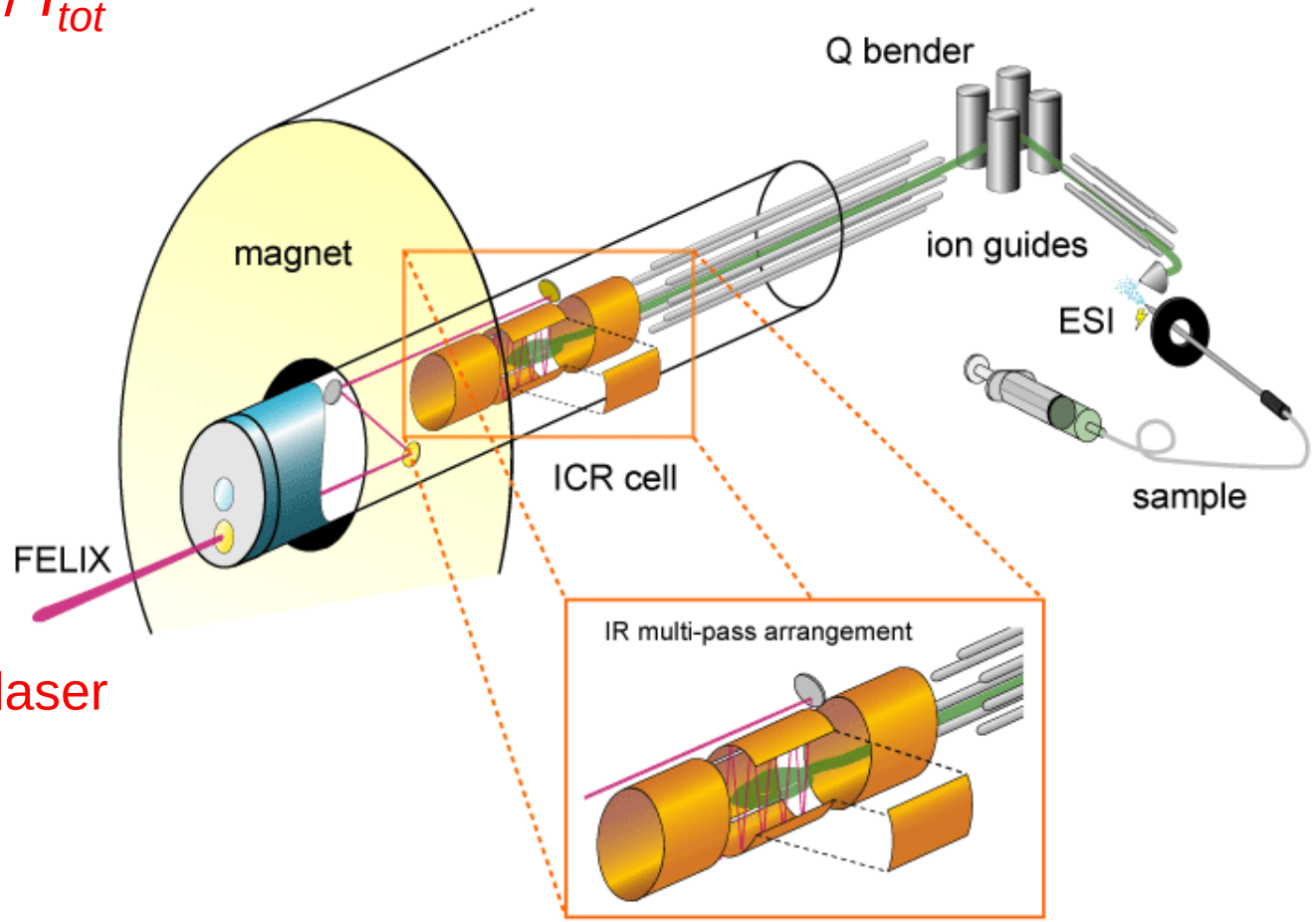
- Gas-phase conformations and energetics of protonated form of pdAdo and pAdo, $[pdAdo+H]^+$ and $[pAdo+H]^+$
- Smallest unit of DNA and RNA nucleotides: readily provides insight of the effects of protonation on larger nucleotide chains: nucleobase orientation, sugar puckering
- Comparison to previous parallel study of protonated adenine nucleosides, elucidate effects of the phosphate moiety

Wu, R. R.; Yang, B.; Berden, G.; Oomens, J.; Rodgers, M. T. *J. Phys. Chem. B* **2015**, *119*, 2795.

IRMPD Spectroscopy

IRMPD Yield = $\sum I_P / I_{tot}$

$I_{tot} = (I_R + \sum I_P)$



free electron or OPO laser

ESI solution conditions:

pdAdo, pAdo: ~0.1–0.5mM

HCl: ~1.0–3.0 mM

MeOH:H_2O: 50%:50%

Polfer, N. C.; Oomens, *J. Phys. Chem. Phys.* **2007**, *9*, 3804.

Theoretical Methods

- Simulated annealing (Amber 2, 300 cycles at 1000 K)

  ⇒ Candidate structures for higher level optimization.

- Gaussian 09, DFT

  - Optimization and vibrational frequency analyses:

    B3LYP/6-311+G(d,p)

    ⇒Low-energy conformations

    ⇒Molecular parameters

    (vibrational frequencies, intensities, rotational constants)

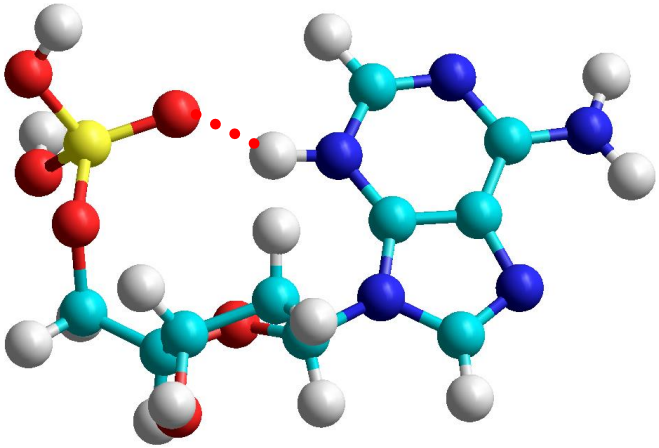
    ⇒IR absorption spectrum

  - Single point energies:

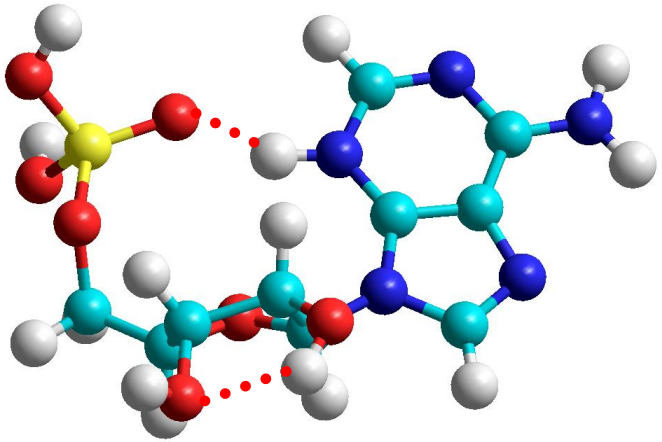
    B3LYP/6-311+G(2d,2p), MP2(full)/6-311+G(d,p)

    ⇒ Energetics/Relative stabilities

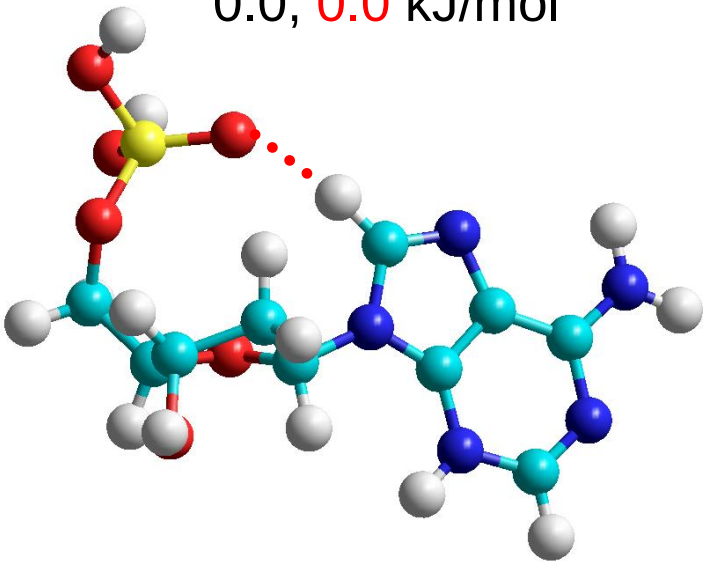
Structures of $[pdAdo+H]^+$ vs. $[pAdo+H]^+$: N3



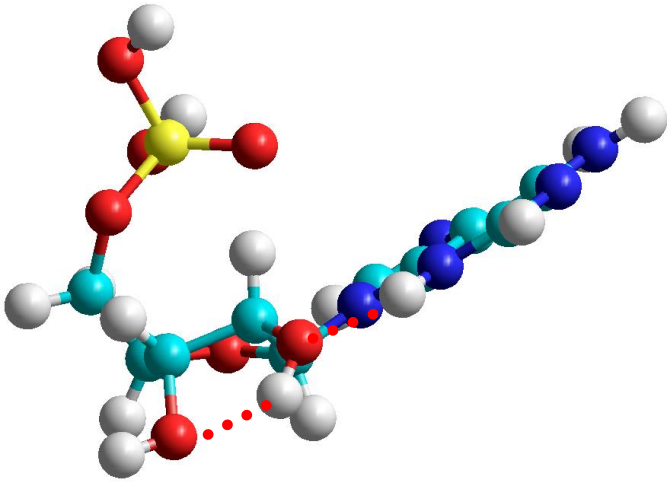
$[pdAdo+H]^+$, N3, syn, C2'-endo
0.0, 0.0 kJ/mol



$[pAdo+H]^+$, N3, syn, C2'-endo
0.0, 0.0 kJ/mol



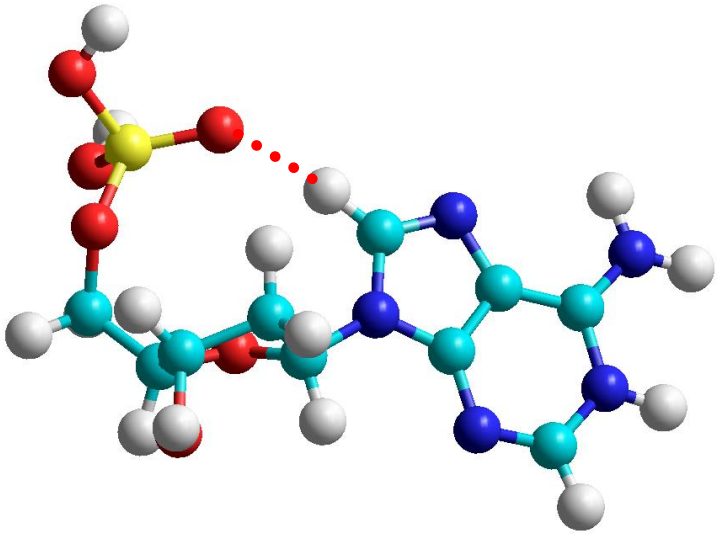
$[pdAdo+H]^+$, N3, anti, C2'-endo
42.6, 45.7 kJ/mol



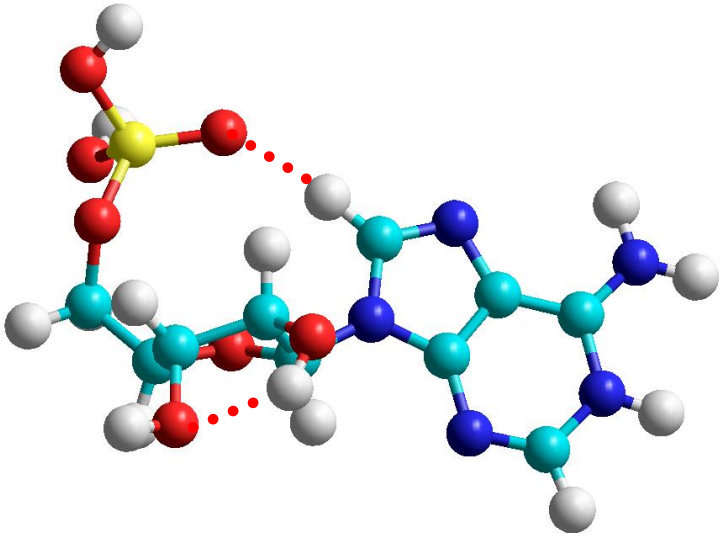
$[pAdo+H]^+$, N3, anti, C2'-endo
25.3, 12.8 kJ/mol

B3LYP, MP2

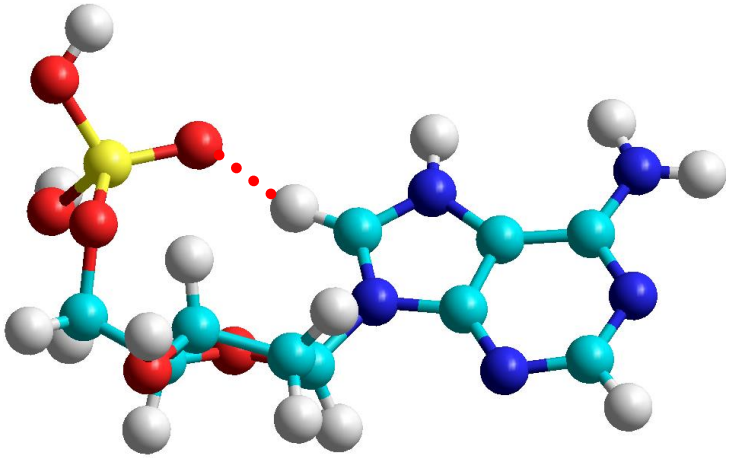
Structures of $[pdAdo+H]^+$ vs. $[pAdo+H]^+$: N1 and N7



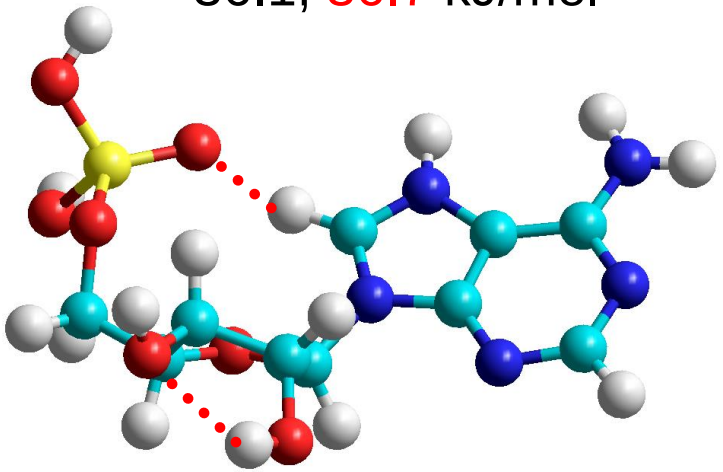
$[pdAdo+H]^+$, N1, anti, C2'-endo
37.2, 38.7 kJ/mol



$[pAdo+H]^+$, N1, anti, C2'-endo
36.1, 36.7 kJ/mol



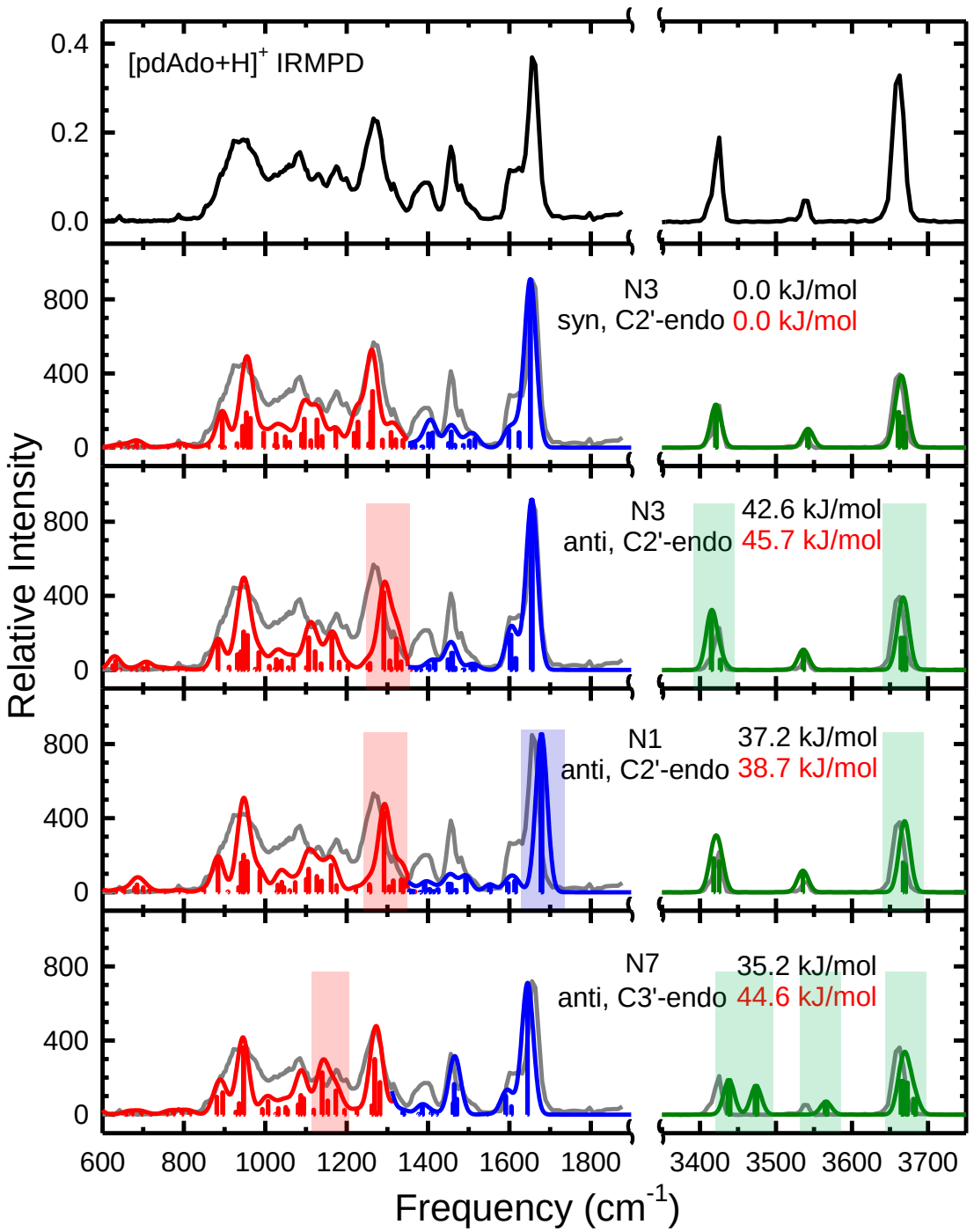
$[pdAdo+H]^+$, N7, anti, C3'-endo
35.2, 44.6 kJ/mol

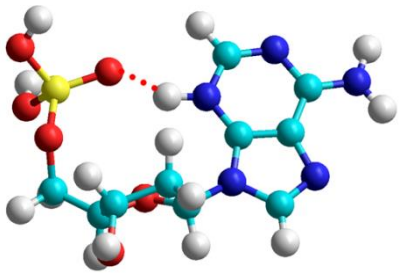


$[pAdo+H]^+$, N7, anti, C3'-endo
41.7, 46.7 kJ/mol

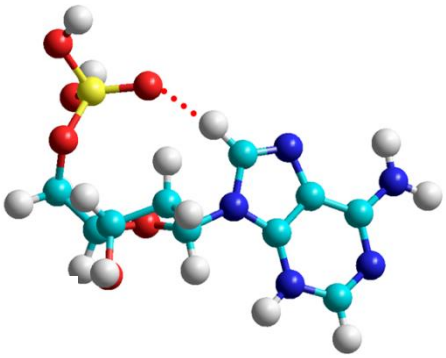
B3LYP, MP2

Comparison of Measured and Calculated IR Spectra

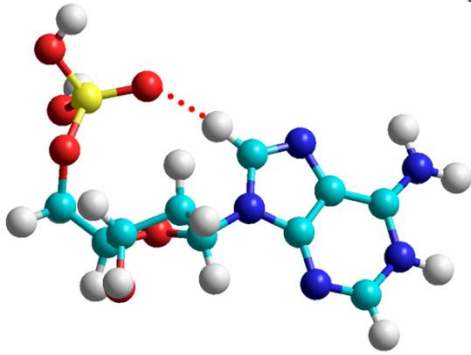




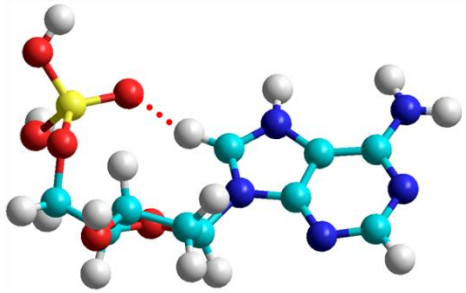
N3, syn, C2'-endo
0.0, 0.0 kJ/mol



N3, anti, C2'-endo
42.6, 45.7 kJ/mol

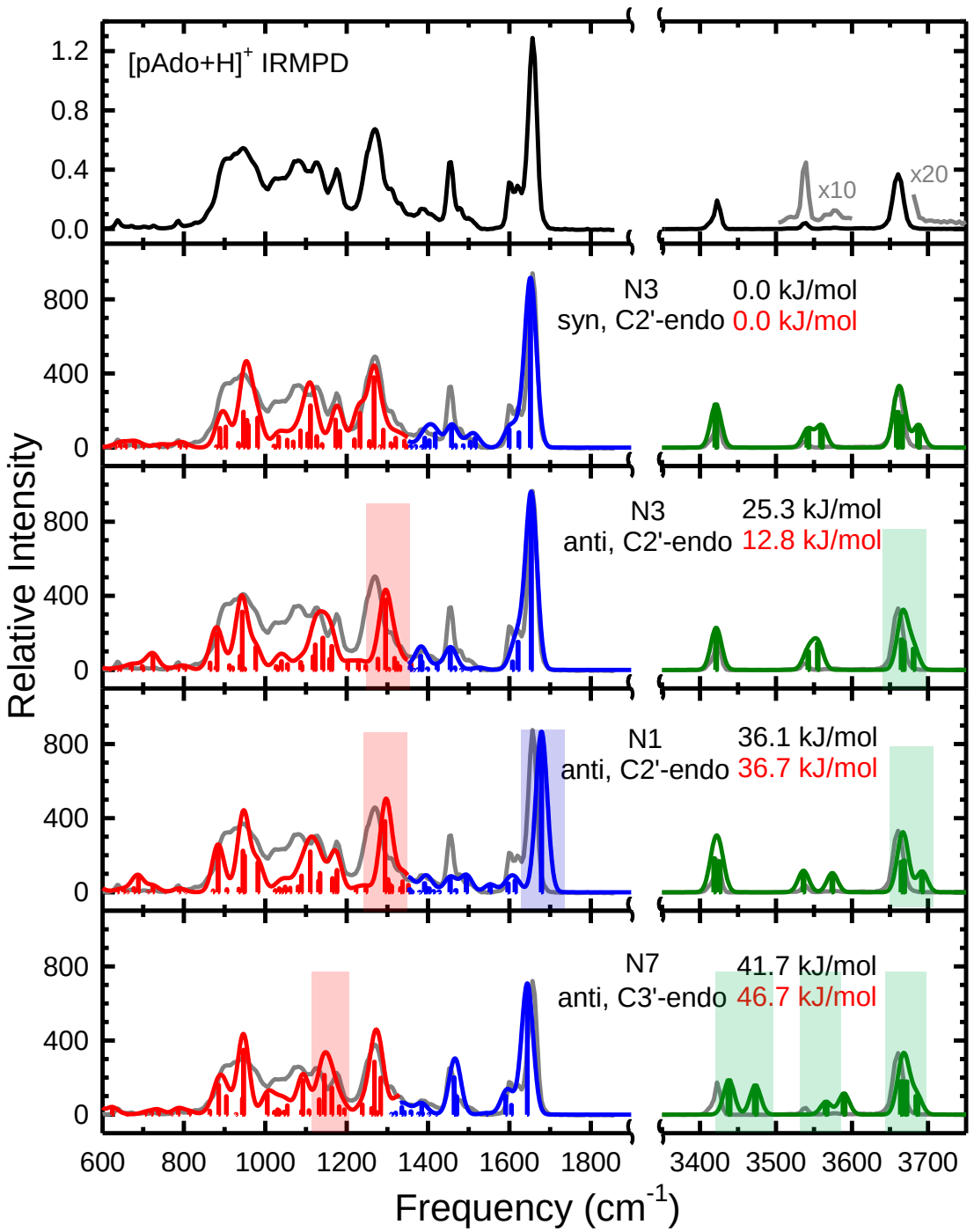


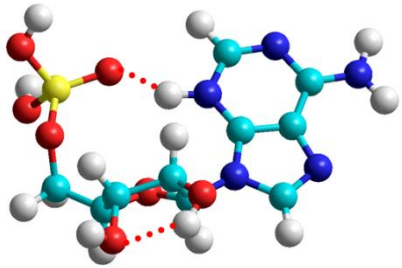
N1, anti, C2'-endo
37.2, 38.7 kJ/mol



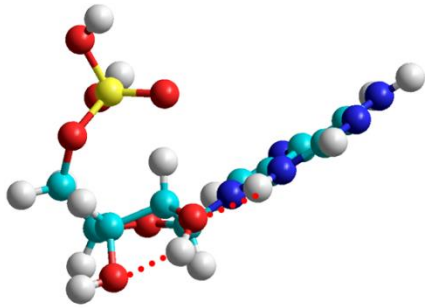
N7, anti, C3'-endo
35.2, 44.6 kJ/mol

Comparison of Measured and Calculated IR Spectra

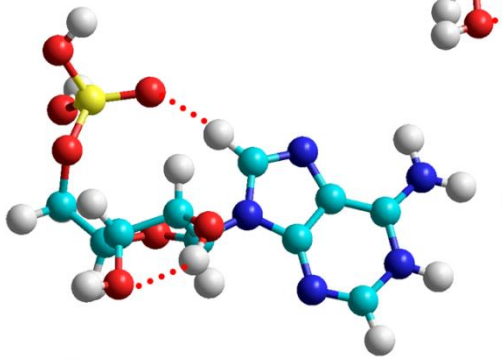




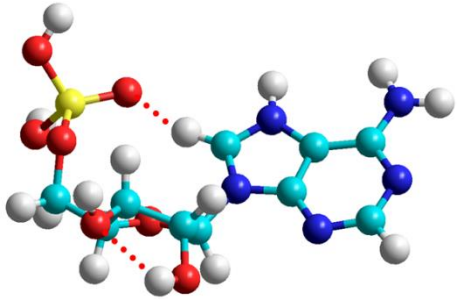
N3, syn, C2'-endo
0.0, 0.0 kJ/mol



N3, anti, C2'-endo
25.3, 12.8 kJ/mol

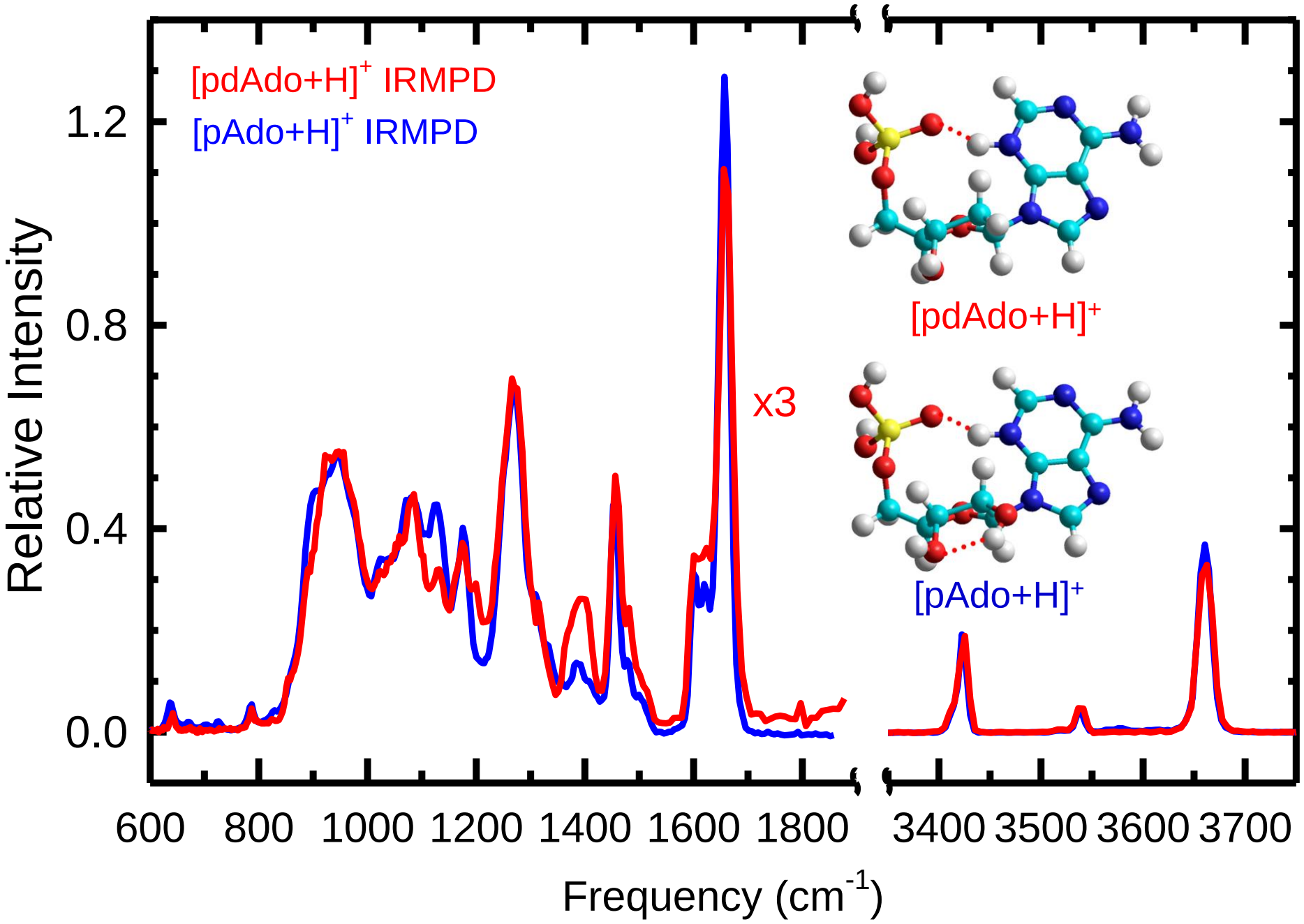


N1, anti, C2'-endo
36.1, 36.7 kJ/mol

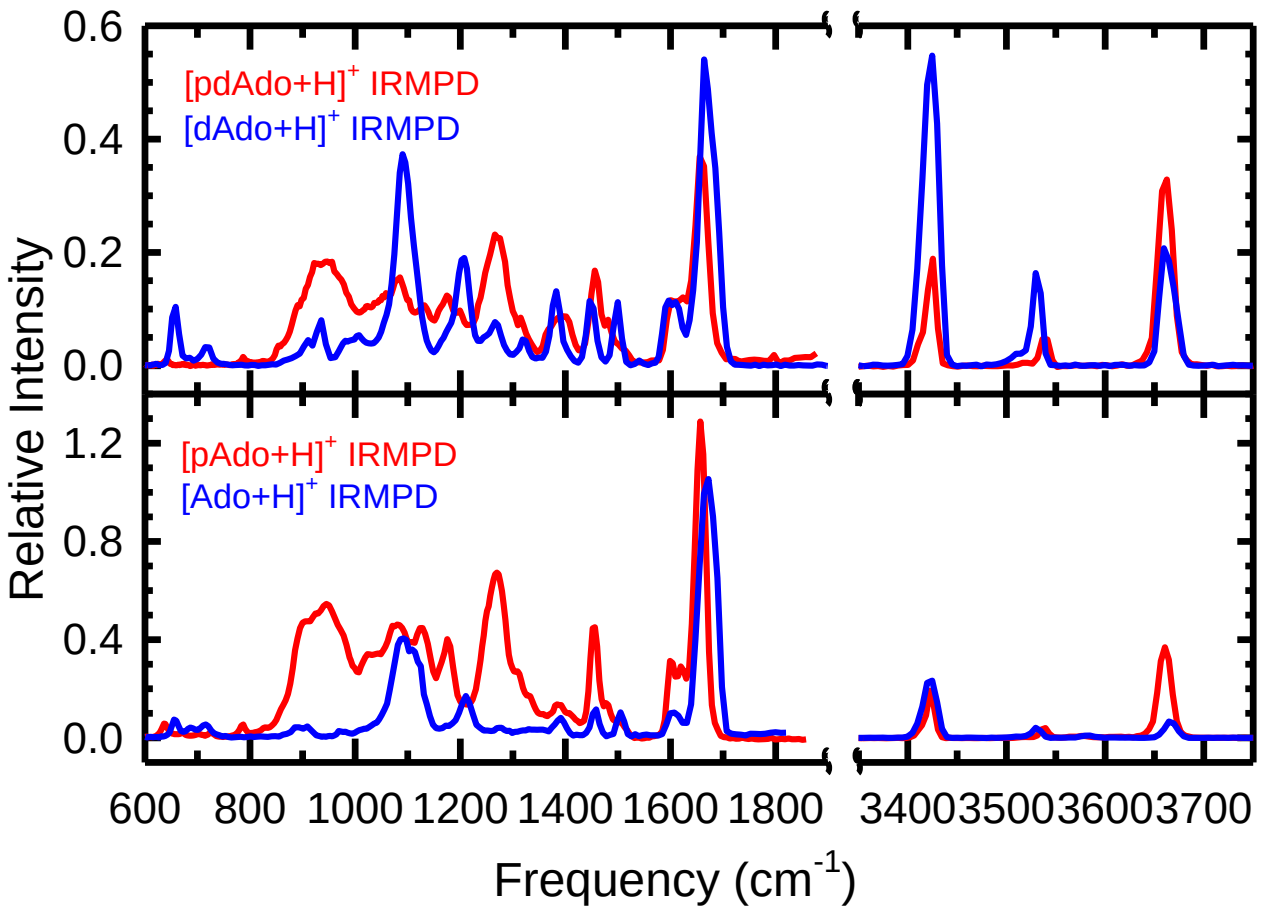


N7, anti, C3'-endo
41.7, 46.7 kJ/mol

[pdAdo+H]$^+$ vs. [pAdo+H]$^+$: Effects of 2'-Hydroxyl

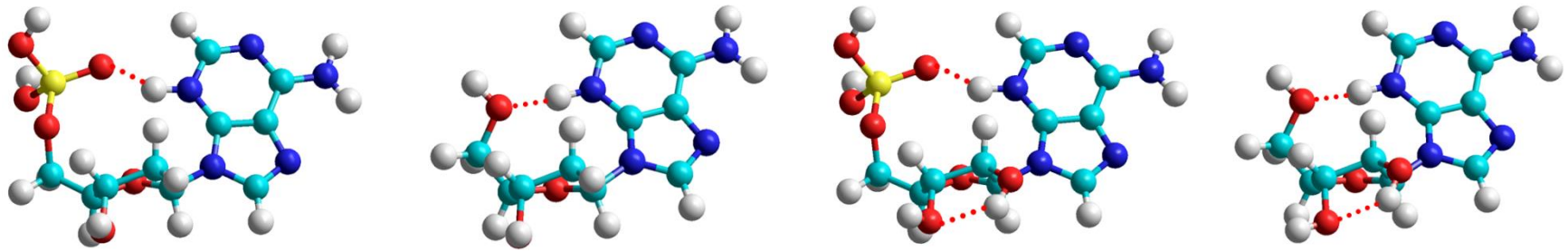


[(d)Ado+H]$^+$ vs. [p(d)Ado+H]$^+$: Effects of Phosphate



Phosphate significantly affects IR features in the IR fingerprint region.

IRMPD band intensities of [pdAdo+H]$^+$ and [pAdo+H]$^+$ higher than those of [dAdo+H]$^+$ and [Ado+H]$^+$ or IRMPD bands observed only for [pdAdo+H]$^+$ and [pAdo+H]$^+$ are likely due to the presence of phosphate moiety.



[pdAdo+H]$^+$ [dAdo+H]$^+$ [pAdo+H]$^+$ [Ado+H]$^+$

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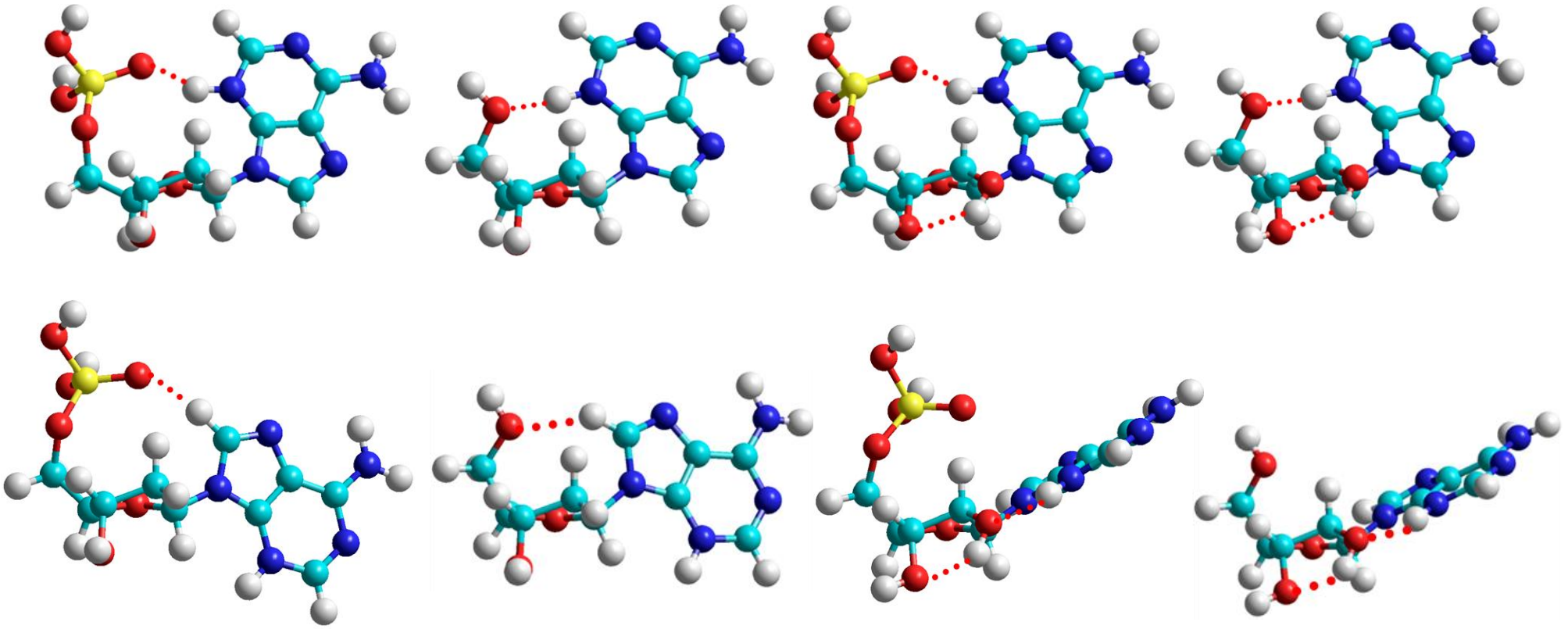
[(d)Ado+H]$^+$ vs. [p(d)Ado+H]$^+$: Effects of Phosphate



42.6, 45.7 kJ/mol

[pdAdo+H]$^+$

28.3, 35.3 kJ/mol

[dAdo+H]$^+$

25.3, 12.8 kJ/mol

[pAdo+H]$^+$

4.7, 7.0 kJ/mol

[Ado+H]$^+$

N3H$^+$···OP–OH is stronger than N3H$^+$···O5'H

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Conclusions

- Stabilities of the gas-phase conformations of $[pdAdo+H]^+$ and $[pAdo+H]^+$ are strongly dependent on protonation sites.
- N3, most favorable site of protonation:
  - syn nucleobase orientation, $N3H^+\cdots O{=}P{-}OH$ stablization
  - highly parallel to $[dAdo+H]^+$ and $[Ado+H]^+$: N3, syn, $N3H^+\cdots OH$
- Effects of 2'-hydroxyl:
  - O2'H···O3'H: more anharmonic, leads to shift of O3'H stretching
  - O2'H···O3'H stabilizes sugar moiety and much higher IRMPD yield is observed for $[pAdo+H]^+$ in IR fingerprint region, does not affect the IRMPD yield in the hydrogen-stretching region.
- $[(d)Ado+H]^+$ vs. $[p(d)Ado+H]^+$:
  - Broadening of IR features below ~1400 cm^{-1}.
  - IRMPD bands only observed for $[p(d)Ado+H]^+$ in IR fingerprint region, leads to vibrational stretches of phosphate moiety
  - Highly parallel IRMPD profiles in the hydrogen-stretching region
  - Higher IRMPD band intensities at ~3665 cm^{-1} for $[p(d)Ado+H]^+$

Acknowledgement

The People:

- Dr. M. T. Rodgers
- Rodgers Group, Dr. C. E. Frieler.
- Computational Resource: WSU C&IT
- FELIX facility staff.

Financial Support:

National Science Foundation

OISE-0730072, CHE-1409420

Department of Chemistry

WAYNE STATE UNIVERSITY

- **Thomas C. Rumble Fellowship**
- **David F. Boltz Analytical Award**
- **Summer Dissertation Fellowship**